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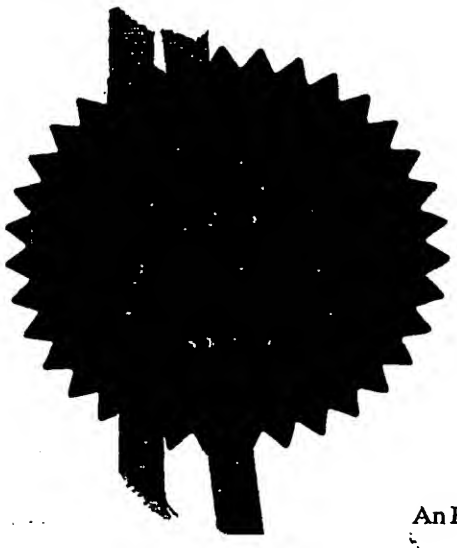

Signed P. Mahoney

Dated 4 March 2003

GB 0200507.2

By virtue of a direction given under Section 30 of the Patents Act 1977, the application is proceeding in the name of

IMPERIAL COLLEGE INNOVATIONS LIMITED,
Sherfield Building,
Exhibition Road,
South Kensington,
LONDON,
SW7 2AZ,
United Kingdom

Incorporated in the United Kingdom,

[ADP No. 07409436002]

and

OREGON HEALTH AND SCIENCE UNIVERSITY, A PUBLIC INSTITUTION OF THE STATE OF OREGON, U.S.A,
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United States of America

[ADP No. 08561870001]

Patents Form 1/77

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1. Your reference

JSvn/746DIAN02 E686959-1 D00001
P01/7700 0.00-0200507.2

2. Patent application number
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0200507.2

10 JAN 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

SECTION 30 (1977 ACT) APPLICATION FILED 28/1/03

IMPERIAL COLLEGE INNOVATIONS LIMITED
~~4 Prince's Gate~~ Sherfield Building
~~Exhibition Road~~ London
~~London SW7 2QA~~ SW7 2AZ
~~England~~

See AIL 2447102

Patents ADP number *(if you know it)*

~~75543478001~~
7409436002

If the applicant is a corporate body, give the country/state of its incorporation

England

4. Title of the invention

APPETITE SUPPRESSION

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ABEL & IMRAY
20 Red Lion Street
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England

Patents ADP number *(if you know it)*

174001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
	n/a	

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

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11. I/We request the grant of a patent on the basis of this application.

Signature Abel & Imray Date 10 Jan 2001

Abel & Imray

12. Name and daytime telephone number of person to contact in the United Kingdom Judith Silveston 0207 242 9984

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APPETITE SUPPRESSION

INTRODUCTION

The present invention relates to appetite control and control of feeding and food intake, especially in humans.

BACKGROUND OF THE INVENTION

A large proportion of people in the developed world may be classified as overweight. The term "overweight" when used in relation to a person denotes a weight greater than the recommended maximum weight for that person. Commonly this is indicated by a Body Mass Index (BMI) of over 25. Body Mass Index is calculated as weight in kilograms divided by height in meters^2. The term "overweight" encompasses a wide range, from slightly overweight to clinically obese, which is generally considered to be BMI of over 30. Excess weight, in particular clinical obesity, may cause or contribute to a wide range of medical conditions. It is desirable that excess weight should be lost and that a weight within the recommended range should be achieved and maintained. Furthermore, people with weight within the recommended range, for example, in the upper part of the recommended range, may wish to reduce their weight, to bring it closer to the mid-range.

Although many investigations have been carried out, the mechanism of normal physiological control of appetite and feeding has remained elusive. The orexigenic neuropeptide Y (NPY) and the anorectic alpha melanocortin stimulating hormone (αMSH) systems of the hypothalamic circuits of the arcuate nucleus appear to be important in the central regulation of appetite[1,15]. However, the mechanisms that signal ingestion of food directly to the hypothalamus are

not clear. Leptin has been proposed to fulfil this role[2]. However, the role of leptin in everyday physiological control of food intake is not clear.

A number of peptide hormones are secreted by the cells of the gastrointestinal tract in response to food intake, including cholecystokinin, glucagon-like peptides and polypeptide YY. One of the glucagon-like peptides, glucagon-like peptide-1 (GLP-1) has been proposed for use in human appetite control, see WO 99/47161. However the levels of GLP-1 required to produce an effect on appetite are higher than levels that generally occur under normal physiological conditions.

Another of the gut hormones, polypeptide YY, called PYY, is found to delay gastric emptying and to enhance nutrient uptake. WO 94/22467 discloses the use of PYY receptor agonists in the treatment of AIDS, especially during cachexia (wasting). WO 96/22783 discloses the use of PYY and PYY receptor agonists to enhance gastrointestinal absorption of nutrients, and also the use of PYY and PYY receptor agonists to increase weight gain in a subject, both in animals, for commercial reasons, and in humans, for therapeutic reasons. WO 96/22783 also discloses the converse effect, that of using PYY or PYY receptor antagonists to reduce nutrient uptake, for example, in chronic obesity.

SUMMARY OF THE INVENTION

The present invention is based on the surprising observation that, contrary to the previous disclosures of the use of PYY to increase weight gain, PYY 3-36, the major circulating form of polypeptide PYY, reduces food

intake and weight gain. Further investigations indicate that PYY 3-36 is acting via the neuropeptide Y Y2 receptor in the brain. PYY 3-36 is a high affinity agonist at the neuropeptide Y Y2 receptor[8].

Accordingly, the present invention relates the use of a PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in the control of food intake in a mammal, in particular in the reduction or inhibition of food intake. The invention also relates to the use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in weight control and treatment, reduction or prevention of obesity, in particular any one or more of the following: preventing and reducing weight gain; inducing and promoting weight loss; and reducing obesity as measured by the Body Mass Index. The invention further relates to the use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in control of any one or more of appetite, satiety and hunger, in particular any one or more of the following: reducing, suppressing and inhibiting appetite; inducing, increasing, enhancing and promoting satiety and sensations of satiety; and reducing, inhibiting and suppressing hunger and sensations of hunger. The invention relates to the use of PYY, PYY 3-36 or another neuropeptide Y Y2 agonist in maintaining any one or more of a desired body weight, a desired Body Mass Index, a desired appearance and good health.

The present invention also relates the use of a neuropeptide Y Y2 receptor antagonist in the control of food intake in a mammal, in particular to increase, promote or stimulate food intake. The invention also relates to the use of a neuropeptide Y Y2 receptor antagonist in

weight control and treatment or prevention of wasting or anorexia, in particular any one or more of the following: inducing, promoting and increasing weight gain; reducing, inhibiting and preventing weight loss; and increasing body mass as measured by the Body Mass Index. The invention further relates to the use of a neuropeptide Y Y2 receptor antagonist in control of any one or more of appetite, satiety and hunger, in particular any one or more of the following: increasing, inducing and promoting appetite; reducing, inhibiting or preventing satiety and sensations of satiety; and increasing, promoting and enhancing hunger and sensations of hunger.

The various uses of the neuropeptide Y Y2 receptor agonist set out above may be in a method of treatment of a mammalian subject in need of such treatment, or may be in the manufacture of a medicament for such treatment. The neuropeptide Y Y2 receptor agonist or antagonist should be administered in an amount effective to achieve the stated object. Some of the treatments described above are medical treatments, for example, the treatment of obesity. Others, however, do not relate to medical treatment, and are part of the maintenance of a healthy lifestyle, or are for cosmetic purposes.

The mammal is generally a human but may be a commercially reared animal or a companion animal.

It will, of course, be understood that if the treatment involves weight loss or reduction of weight gain, whether in the treatment of obesity or otherwise, the treatment should preferably be part of a calorie-controlled diet.

Increased weight gain may be desirable for commercial reasons in animal husbandry. As regards humans, companion animals and other objectively or subjectively valuable animals, for example, horses, it is desirable to stimulate appetite and increase weight gain when appetite is poor and weight is lost or may be lost, for example, during illness, after accidental or surgical trauma, for example, burns, and especially severe burns, during convalescence, in the elderly, and in anorexia and bulimia, and in other wasting conditions. Appetite stimulation and increase in weight may be particularly desirable in specific conditions, for example, during cachexia (wasting) in AIDS, and in cancer patients.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Feeding response to PYY 3-36 in rats.

Figure 1a: Dark-phase feeding: Food intake after intraperitoneal injection of PYY 3-36. Freely feeding rats were injected with PYY 3-36 at the doses indicated (μg/100g), or saline, just prior to 'lights off' and 4 hour cumulative food intake was measured. Results are the mean ± s.e.m. (n = 8 per group), * = $p < 0.05$, ** = $p < 0.01$, *** = < 0.001 compared to saline.

Figure 1b: Food intake after intraperitoneal injection of PYY 3-36. Fasted rats were injected with PYY 3-36 at the doses indicated (μg/100g), or saline, and 4 hour cumulative food intake was measured. Results are the mean ± s.e.m. (n = 8 per group), * = $p < 0.05$, ** = $p < 0.01$, *** = < 0.001 compared to saline.

Figure 1c: Cumulative food intake after intraperitoneal injection of saline or PYY 3-36. Fasted rats were injected with either saline (closed bars) or PYY 3-36 5μg/100g (open bars) and cumulative food intake measured at the time points indicated. Results are expressed as mean ± s.e.m. (n = 12 per group), ** = $p < 0.01$ compared to saline.

Figure 1d: Body weight gain during chronic treatment with PYY 3-36. Rats were injected intraperitoneally with PYY 3-36 5μg/100g (open squares) or saline (filled inverted triangles) twice daily for 7 days. Body weight gain was calculated each day. Results are expressed as mean ± s.e.m. (n = 12 per group) ** = $p < 0.01$ compared to saline.

Figure 2. C-fos expression in POMC-EGFP mice.

Figures 2a and 2b: C-fos expression in the arcuate nucleus of POMC-EGFP mice in response to intraperitoneal PYY 3-36 (2μg/100g in 100μl) or saline. Scale bar 50μm.

Figure 2c: An example of colocalisation of POMC-directed EGFP fluorescence and c-fos immunostaining.

Figure 3. Intra-arcuate PYY 3-36 in rats and feeding effects of IP PYY 3-36 in Y2R null mice.

Figure 3a: Food intake following intra-arcuate PYY 3-36 injection: Food intake following intra-arcuate injection of PYY 3-36. Fasted rats were injected with saline or PYY 3-36 into the arcuate nucleus at the doses indicated. Post-injection 2 hour food intake was measured, ** = $p < 0.01$ compared to saline.

Figures 3b and 3c: Feeding response to PYY 3-36 in Y2R null mice: wild type littermates mice (b) and Y2R null mice (c),

fasted for 24 hours, were injected with PYY 3-36 at the doses indicated (μg/100g), or saline, and 4-hour cumulative food intake was measured. Results are the mean ± s.e.m. (n = 5 per group), * = $p < 0.05$, ** = $p < 0.01$ compared to saline.

Figure 4. Electrophysiological and neuropeptide responses to PYY 3-36 and Y2A.

Figure 4a: Effect of PYY 3-36 (10 nM) on POMC neuron membrane potential (figure representative of recordings from 22 neurons).

Figure 4b: Effect of PYY 3-36 (50 nM) on spontaneous IPSCs onto POMC neurons (figure representative of recordings from 13 neurons).

Figures 4c and 4d: αMSH (c) and NPY (d) released from hypothalamic explants in response to Y2A [N-acetyl-(Leu^{28},Leu^{31})NPY(24-36)]. Hypothalamic slices were incubated with artificial CSF, with or without 50 nM Y2A, for 45 min. Results are expressed as mean ± s.e.m. (n = 40), *** = $p < 0.001$ compared to saline.

Figure 5. Effect of PYY 3-36 infusion on appetite and food intake in human subjects.

Figure 5a: Calorie intake from "free-choice" buffet meal 2 hours after infusion with saline or PYY 3-36. The thin lines indicate individual changes in calorie intake for each subject between saline and PYY 3-36 administration. The thick line represents mean change between the two infusions (n = 12).

Figure 5b: 24 hour calorie intake following infusion with saline or PYY 3-36. Total calorie intake, as assessed by food diaries, is shown for the 24-hour period following either saline or PYY 3-36 infusion. Data is given as mean ± s.e.m. (n = 12), *** = $p < 0.0001$ compared to saline.

Figure 5c: Appetite score (relative scale). Visual analogue scores[17] show perceived hunger during and after infusions. The results are presented as change from baseline scores and are the mean ± s.e.m. for all 12 subjects.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates the use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in the control of food intake in a mammal, in particular in the reduction or inhibition of food intake. The invention also relates to the use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in weight control and treatment, reduction or prevention of obesity, in particular any one or more of the following: preventing and reducing weight gain; inducing and promoting weight loss; and reducing obesity as measured by the Body Mass Index. The invention further relates to the use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist in control of any one or more of appetite, satiety and hunger, in particular any one or more of the following: reducing, suppressing and inhibiting appetite; inducing, increasing, enhancing and promoting satiety and sensations of satiety; and reducing, inhibiting and suppressing hunger and sensations of hunger.

The present invention also relates the use of a neuropeptide Y Y2 receptor antagonist in the control of food intake in a mammal, in particular to increase, promote or stimulate food intake. The invention also relates to the use of a neuropeptide Y Y2 receptor antagonist in weight control and treatment or prevention of wasting or anorexia, in particular any one or more of the following: inducing, promoting and increasing weight gain; reducing, inhibiting and preventing weight loss; and increasing body mass as measured by the Body Mass Index. The invention further relates to the use of a neuropeptide Y Y2 receptor antagonist in control of any one or more of appetite, satiety and hunger, in particular any one or more of the following: increasing, inducing and promoting appetite; reducing, inhibiting or preventing satiety and sensations of satiety; and increasing, promoting and enhancing hunger and sensations of hunger.

PYY, PYY 3-36 or any other neuropeptide Y Y2 receptor agonist may be used in the present invention. Examples of such compounds include peptide and non-peptide agonists, for example, the highly selective agonist N-acetyl-(Leu^{28},Leu^{31})NPY(24-36), which is called Y2A.

The neuropeptide Y Y2 receptor (NPY Y2 receptor) agonist is, in particular, peptide YY (PYY) or a derivative, analogue, fragment or mimetic thereof that has NPY Y2 receptor agonist activity, especially NPY Y2 receptor agonist activity analogous to that of PYY or PYY 3-36. The NPY Y2 receptor agonist is especially PYY 3-36, the major circulating form of PYY, or a derivative, analogue, fragment or mimetic of PYY 3-36 that has NPY Y2 receptor agonist activity, especially NPY Y2 receptor agonist

activity analogous to that of PYY 3-36. Analogues of PYY and PYY 3-36 include peptide analogues. The NPY Y2 receptor agonist may be a non-peptide mimetic of PYY or PYY 3-36.

Peptide YY (PYY) is a 36 amino acid peptide amide. The amino acid sequences of human and porcine PYY are as follows:

Human PYY:
YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY (SEQ.ID.NO.:1)

Porcine PYY:
YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY (SEQ.ID.NO.:2)

In vivo PYY is produced by "L type" endocrine cells of the gastrointestinal tract and pancreas. The first two residues are cleaved to give PYY 3-36, which is the major circulating form. In the present invention PYY or PYY 3-36 may be used, PYY 3-36 being preferred. The PYY or PYY 3-36 is preferably human, but may be PYY or PYY 3-36 of any other animal, for example, porcine PYY or PYY 3-36. The PYY or PYY 3-36 may be obtained from a human or animal source, for example, from extracts of gut, or from cultured gut endocrine cells. Preferably the PYY or PYY 3-36 is produced recombinantly or by chemical synthesis.

The term "human PYY" is used to denote any PYY molecule having the amino acid sequence of human PYY and includes recombinant and synthetic PYY having the amino acid sequence of human PYY. The same nomenclature is used in relation to PYY 3-36 and derivatives and analogues of PYY

and PYY 3-36 and in relation to corresponding molecules of other animal origin.

It may be particularly advantageous to use PYY or PYY 3-36 according to the present invention because PYY is a naturally-occurring, physiologically relevant agent and can be used at normal physiological levels, thus reducing the likelihood of adverse side effects and enabling safe long-term use, if required.

A derivative, analogue, fragment or mimetic of PYY or of PYY 3-36 for use according to the present invention must have NPY Y2 receptor agonist properties, and preferably has NPY Y2 receptor agonist properties similar to those of PYY or PYY 3-36, for example, similar properties in relation to binding to the NPY Y2 receptor. The more similar the agonist properties to those of PYY or PYY 3-36, the better. Methods for determining the ability of a compound to act as an NYP Y2 receptor agonist are known, see for example, WO94/22467. Details of the experimental procedures are given in the references cited above. It is generally preferable to use an agonist that is selective for the NPY Y2 receptor, that it to say, it shows little activity in relation to the other NPY receptors, for example, Y1 and Y5 or for other receptors found in the hypothalamus.

A derivative, analogue, fragment or mimetic of PYY or of PYY 3-36 for use according to the present invention should also have the ability to reach the relevant part of the brain, for example, the arcuate nucleus, in particular from the circulation. The agent used should also be sufficiently stable and long-lived in vivo to have a biological effect and should be non-toxic at the doses used.

An active fragment of PYY or of PYY 3-36 is a truncated version of the molecule that retains the agonist properties. Such an active fragment may be more selective for the NPY Y2 receptor than the parent molecule. A derivative of PYY or of PYY 3-36 or of a fragment thereof may be formed, for example, by modifying the natural amino acid sequence, by modifying the N- or C-terminal group, or by modifying one or more side chains. The term derivative includes fusion proteins that comprise a PYY or modified PYY sequence and a non-PYY sequence, and also includes salts with acids and bases and also Zwitterionic salts, especially physiologically tolerable salts.

An analogue of PYY or of PYY 3-36 or of a fragment thereof is a compound comprising an amino acid sequence which, while not having the amino acid sequence of PYY, PYY 3-36 or a fragment thereof, is has the NPY Y2 agonist properties of PYY or of PYY 3-36. An example of a PYY analogue is N-acetyl(Leu^{28},Leu^{31})NPY(24-36), which is a highly selective NPY Y2 agonist.

A PYY or PYY 3-36 mimetic is any compound, especially a non-peptide compound, that has the NPY Y2 receptor agonist properties of PYY or PYY 3-36. Compounds that fall into this category may be identified by screening against the NPY Y2 receptor, for example, using the cloned receptor. Such screening methods are well known per se and are particularly useful for screening synthetic libraries of chemical compounds, for example, produced by combinatorial chemistry.

Analogues, derivatives and mimetics that have are more stable, more active, more selective or more resistant to enzymatic degradation than PYY or PYY 3-36, or which have more than one of such properties, are particularly preferred.

WO94/22467 discloses analogues of PYY, PYY 3-36 and fragments thereof, methods for producing the analogues and pharmaceutical compositions comprising the analogues. Any of those analogues or pharmaceutical compositions may be used in the present invention. WO96/22783 describes in general terms methods for designing and producing PYY analogues and mimetics. Such methods may be used to obtain PYY analogues and mimetics for use in the present invention.

For use according to the present invention, the NPY Y2 agonist should generally be presented in admixture with a pharmaceutically suitable carrier in the form of a pharmaceutical composition. Examples of such compositions and routes of administration are given in WO 94/22467 and WO96/22783.

Many of the NPY Y2 receptor agonists described above, including PYY and PYY 3-36, are peptides. As such molecules are digested in the gastro-intestinal tract, it is preferable to administer such compounds by a non-oral route. Examples of parenteral routes are injection and infusion, for example, subcutaneous, intravenous, intramuscular and intradermal injections, and nasal and topical administration. Rectal administration may be used. If an NPY Y2 receptor agonist is not a peptide, it may be administered orally, including by buccal or sublingual

administration. An alternative for peptide agonists is to treat the peptide such that it is resistant to digestion in the gut. Oral routes may then be used.

For administration by injection or infusion, the carrier is, for example, sterile, pyrogen-free water (water for injection), or a saline solution, for example, phosphate buffered saline. For nasal administration, the NYP Y2 receptor agonist may be formulated in a liquid that can be administered as drops or as a spray. An aerosol formulation may be used for administration by inhalation. For topical administration, a cream, gel or ointment formulation may be used, or the agonist may be administered using an adhesive "patch". Suppositories are generally used for rectal administration of active substances, with enema solutions as an alternative. Formulations for oral administration include tablets, pills and capsules. Liquid formulations for oral administration include syrups. The NYP Y2 receptor agonist may be administered in a delayed release form, either an oral delayed form, for example, a coated tablet, for example, an enteric coated tablet, or as a depot formulation for administration, for example, subcutaneously. Suitable pharmaceutical formulations are fully described in the literature of that art, for example, in Remington's Pharmaceutical Sciences by EW Martin. In all cases a pharmaceutical composition should contain an effective amount of the NPY Y2 agonist. The same considerations apply to NYP Y2 receptor antagonists.

PYY or PYY 3-36 is generally administered by injection or infusion, for example, by a bolus injection or using a continuous or discontinuous pump system, for example, as

developed for administration of insulin to diabetic subjects.

The dose of PYY or PYY 3-36 is preferably based on the physiological levels observed post-prandially[5-7]. The normal circulating levels of PYY 3-36 are about 8 pmol/litre, typically rising to about 40 to 60 pmol/litre after a meal. Other neuropeptide Y Y2 agonists may be used at analogous doses. A single dose may be administered per day, or divided doses may be used. It may be preferable to administer a dose of the NPY Y2 agonist before and/or after each meal to simulate normal PYY 3-36 levels. A discontinuous pump may be particularly useful for such a regime. It is one of the advantages of the present invention that a naturally occurring peptide, PYY or PYY 3-36 may be used for its own in vivo physiological effect. This results in minimal side effects and enables long term use, if necessary.

Neuropeptide Y Y2 receptor antagonists, used according to the invention for various indications relating to increasing weight or reducing weight loss, as described above, are known or may be identified by screening against the NPY Y2 receptor, see WO94/22467 and WO96/22783. Suitable pharmaceutical formulations and routes for the administration of an NPY Y2 receptor antagonist are as described above for NPY Y2 receptor agonists.

PYY had been proposed previously for increasing weight gain in humans for therapeutic reasons, including the treatment of anorexia and bulima, and in animals for therapeutic and commercial reasons, see WO96/22783.

It has now been found, surprisingly, that peptide PYY and its major circulating form, PYY 3-36 have the opposite effect, causing inhibition of feeding when given both intra-arcuately and peripherally in rodents. Direct intra-arcuate injection of PYY 3-36 was found to inhibit food intake. A single peripheral injection of PYY 3-36 in rats produced long-lasting inhibition of food intake and when administered daily for seven days, resulted in both reduced cumulative food intake and weight loss.

Furthermore, when administered to humans, PYY was found to reduce appetite. When infused into humans at physiological post-prandial levels, PYY3-36 significantly decreased appetite and reduced food intake by a third over 12 hours. Both the effect itself and the duration of the effect are surprising and unpredictable. The effects, produced at physiological levels of the peptide, are strong indications that PYY acts in vivo to regulate feeding behaviour.

It has also been found that peripheral administration of PYY 3-36 in the rat caused an increase of c-fos immunoreactivity in the arcuate nucleus of the hypothalamus and a decrease in hypothalamic neuropeptide Y (NPY) mRNA. Further, electrophysiological studies demonstrated that PYY 3-36 inhibits synaptic activity of the NPY nerve terminals and thus activates POMC neurons, which are known to receive inhibitory NPY synaptic inputs. Furthermore, it has been found that N-acetyl(Leu^{28},Leu^{31})NPY(24-36), which is a highly selective NPY Y2 receptor agonist, also inhibits food intake when injected intra-arcuately.

These results suggest that the gut hormone PYY 3-36 may act via the neuropeptide Y Y2 receptor. This hypothesis is

supported by the observation that when PYY 3-36 was administered to neuropeptide Y Y2 receptor null mice (Y2R gene knock out mice), no inhibition of feeding was observed. Administration of PYY 3-36 to wild type littermates of the Y2R null mice was fully effective in inhibiting feeding.

While not being bound by the following theory, it is proposed that a novel gut-brain pathway that inhibits feeding after meals has been found. The natural pathway involves release of PYY from the gut, its conversion to PYY 3-36, which acts as an agonist on the neuropeptide Y Y2 receptor (NPY Y2 receptor) in the brain. The NPY Y2 receptor acts as a inhibitory pre-synaptic receptor reducing release of neuropeptide Y, which is widely accepted to be the most potent stimulator of feeding, and also acting on the anorexigenic melanocortin systems, the result of the NPY Y2 receptor activity being to suppress appetite and decrease food intake. The action of PYY 3-36 may occur in the arcuate nucleus of the hypothalamus, but other areas may be also be involved.

The results obtained show that PYY 3-36, a gut hormone that circulates in the blood, inhibits appetite at physiological concentrations, and that the inhibitory effect is observed even for some hours after the hormone has been cleared from the blood. This effect has been observed in all species tested, i.e. in mouse, rat and human. The circulating gut hormone appears to act via hypothalamic circuits. The reduction of messenger RNA, necessary for the synthesis of brain appetite regulating hormones, in particular of hypothalamic NPY mRNA may be a possible mechanism for the long action of PYY 3-36.

In summary, the results obtained indicate that PYY 3-36 is a physiologically important everyday regulator of appetite in man. The results also demonstrate that, by administering PYY, PYY 3-36 or another NPY Y2 receptor agonist, both appetite and food intake can be controlled. Accordingly, PYY, PYY 3-36 and other NPY Y2 receptor agonists may be used inter alia in weight control, in particular to bring about or assist in weight loss or to prevent weight gain, whether for therapeutic reasons as in the treatment of obesity or for "cosmetic" or lifestyle purposes. Conversely, neuropeptide Y Y2 antagonists maybe used in cases where it is desired to stimulate appetite or increase weight, for example, in illness or convalescence, after accidental or surgical trauma, for example, burns, during wasting and anorexia, and in the elderly.

It is particularly advantageous that PYY or PYY 3-36 is a naturally-occurring, physiologically relevant agent, and that it can be used at normal physiological levels, thus reducing the likelihood of adverse side effects and enabling safe long-term use, if required.

The following non-limiting Examples illustrate the invention.

EXAMPLES

Materials and Methods

The following materials and methods were used in the Examples 1 to 5 below.

Animals

Male 200-250g, 7 to 8 weeks old Wistar rats (Charles River Laboratories, United Kingdom) were maintained under controlled temperature (21-23 C) and light conditions (lights on 07:00 - 19:00) with *ad libitum* access to water and food (RM1 diet; SDS Ltd., Witham, United Kingdom) except where stated. Arcuate and paraventricular nuclei cannulations and injections were performed as previously described[12, 14, 15]. Correct intranuclear cannula placement was confirmed histologically at the end of each study period[12, 14, 15]. All animal procedures were approved under the British Home Office Animals (Scientific Procedures) Act, 1986. All injection studies on fasting animals were performed in the early light-phase (0800-0900). All dark-phase feeding studies injections were performed just prior to lights off.

Y2R null mice were generated using Cre-lox P mediated recombination which results in the germline deletion of the entire coding region of the Y2 receptor. All mice were maintained on a 50%C57/Bl6 - 50%129SvJ background. Male mice aged 8-12 weeks and between 20-30 g bodyweight were kept under controlled temperature (21-23 C) and light conditions (lights on 06:00 - 18:00) with *ad libitum* access to water and food (Gordon's Speciality Stock feeds) except where stated. All studies were performed in the early light-phase (0700-0800).

Intraperitoneal injections

Rats were accustomed to IP injection by injections of 0.5ml saline on the two days prior to study. For all studies, animals received an IP injection of either PYY 3-36 or saline in 500μl (for rats) or 100μl (for mice).

Electrophysiology

Patch clamp recordings were made from POMC neurons in the hypothalamus of 180 Πm thick coronal slices from POMC-EGFP mice, as previously reported[2]. Vehicle controls were used in this system, previously validated for the electrophysiological actions of neuropeptides[2]. Data were analysed by ANOVA and Wilcoxon Signed Rank Test.

C-fos expression

IP saline or PYY 3-36 (2µg/100g) were administered two hours into the light phase, to adult male Wistar rats and POMC-EGFP mice[2]. Two hours after injection the animals were transcardially perfused with 4% paraformaldehyde under terminal anaesthesia. Brain sections, 30 Πm thick, were incubated for 60 hours, at 4°C, with rabbit c-fos antiserum (Ab-5 rabbit, Oncogene Research Products, final dilution 1:25,000). Sections were then incubated with biotinylated secondary antibody (Vector Laboratories, 1:600) followed by incubation with avidin-biotin conjugated horseradish peroxidase (Vectastain, Vector Laboratories) and stained using a nickel intensified diaminobenzidine reaction. Data were counted from 3 rats in each group. EGFP fluorescence was observed using an FITC filter set.

RNase protection assay

Total RNA was extracted from hypothalami (Trizol, Gibco). RPAs were performed (RPAIII kit, Ambion) using 5µg RNA and probes specific for NPY, ΔMSH and E actin (internal standard). For each neuropeptide, the ratio of the optical density of the neuropeptide mRNA band to that of E actin was calculated. Neuropeptide mRNA expression levels are

expressed relative to saline control (means ± s.e.m. n = 4 per group). The statistical analysis used was ANOVA, with Bonferroni post hoc analysis.

Plasma assays

Human leptin was measured using a commercially available RIA (Linco Research, USA). All other plasma hormone levels were measured using established in-house RIAs[18]. Glucose concentrations were measured using a YSI 2300STAT analyser (Yellow Springs Instruments Inc., Ohio, U.S.A.). Plasma paracetamol levels were measured using an enzymatic colorimetric assay (Olympus AU600 analyser).

Human Studies

PYY 3-36 was purchased from Bachem (California, USA). The Limulus Amoebocyte Lysate assay test for pyrogen was negative and the peptide was sterile on culture. Ethical approval was obtained from the Local Research Ethics Committee (project registration 2001/6094) and the study was performed in accordance with the principles of the Declaration of Helsinki. Subjects gave informed written consent. Each subject was studied on two occasions with at least 1 week between each study. Volunteers filled out a food diary for three days prior to each infusion, and for the following 24 hours. All subjects fasted and drank only water from 8pm on the evening prior to each study. Subjects arrived at 830 am on each study day were cannulated and then allowed to relax for 30 minutes prior to the onset of the study protocol. Blood samples were collected every 30 minutes into heparinised tubes containing 5,000 Kallikrein Inhibitor Units (0.2ml) of aprotinin (Bayer) and

centrifuged. Plasma was separated and then stored at -70 C until analysis. Subjects were infused with either saline or 0.8 pmol.kg^{1}.min^{-1} PYY 3-36 for 90 minutes, in a double blind randomised crossover design. Two hours after the termination of the infusion, subjects were offered an excess free-choice buffet meal[18], such that all appetites could be satisfied. Food and water were weighed pre- and postprandially and caloric intake calculated. Appetite ratings were made on 100 mm visual analogue scores (VAS) with the text expressing the most positive and the negative rating anchored at each end[17]. VAS was used to assess hunger, satiety, fullness, prospective food consumption and nausea. Caloric intake following saline and PYY 3-36 were compared using a paired *t* test. The post-prandial response curves were compared by ANOVA using repeated paired measures, with time and treatment as factors.

EXAMPLE 1

Effect of peripherally administered PYY 3-36 on feeding in rats

(a) Dark-phase feeding

The effects of intraperitoneal injection of PYY 3-36 on food intake during dark-phase feeding was investigated.

Freely feeding rats were injected with PYY 3-36 at the doses indicated in Figure 1a (μg/100g), or saline, just prior to 'lights off' and 4 hour cumulative food intake was measured. The results given in Figure 1a show that an intraperitoneal injection of PYY 3-36 to freely feeding rats, prior to the onset of the dark-phase, significantly decreased food intake.

(b) Food intake after intraperitoneal injection of PYY 3-36.

Fasted rats were injected with PYY 3-36 at the doses indicated in Figure 1b (μg/100g), or saline, and 4 hour cumulative food intake was measured. The results given in Figure 1b show inhibition of feeding in the fasting rats, similar to that observed for dark-phase feeding.

The plasma PYY 3-36 levels achieved following IP injection of PYY 3-36 were within the normal post-prandial range (peak PYY 3-36 levels 15 minutes post IP injection of 0.30g/100g = 99.3 ± 10.4 pmol/l vs. peak post-prandial level = 112.1±7.8 pmol/l, n = 8-10 per group), suggesting that physiological concentrations of PYY 3-36 inhibit feeding. (PYY is released from the gastro-intestinal tract post-prandially in proportion to the calorie content of the meal ingested[5-7]). PYY 3-36 did not affect gastric emptying (percentage of food ingested remaining in the stomach at 3 hours: PYY 3-36 = 36 ± 1.9 %, saline = 37.4 ± 1.0 % n = 12)[9].

c. Cumulative food intake after intraperitoneal of PYY 3-36

Cumulative food intake was investigated after intraperitoneal injection of saline or PYY 3-36. Fasted rats were injected with either saline or PYY 3-36 5μg/100g and cumulative food intake measured at the time points indicated in Figure 1c. The results in Figure 1c show that PYY 3-36 administered IP twice daily for 7 days resulted in a reduction in cumulative food intake (7 day cumulative food intake: PYY 3-36 = 187.6 ± 2.7g vs. saline = 206.8 ± 2.3, n = 8 per group, $P < 0.0001$)

d. Body weight gain during chronic treatment with PYY 3-36. Rats were injected intraperitoneally with PYY 3-36 5μg/100g or saline twice daily for 7 days. Body weight gain was calculated each day. The results, which are shown in Figure 1d, show that PYY 3-36 administered IP twice daily for 7 days resulted in decreased body weight gain: PYY 3-36 = 48.2 ± 1.3g vs. saline = 58.7 ± 1.9, n = 8 per group, $P < 0.002$.

EXAMPLE 2

Effect of peripherally administered PYY 3-36 on c-fos expression and NPY mRNA in the arcuate nucleus of rats

To investigate whether the inhibition of food intake demonstrated in Example 1 was via a hypothalamic pathway, c-fos expression in the arcuate nucleus, an important centre of feeding control[1, 2], following a single IP (intra-peritoneal) injection of PYY 3-36, was investigated.

Figures 2a and 2b show c-fos expression in the arcuate nucleus of POMC-EGFP mice in response to intraperitoneal PYY 3-36 (2μg/100g in 100μl) or saline. The results obtained show there was a 2 fold increase in the number of cells positive for c-fos in the lateral arcuate of the rat (PYY 3-36 =168 ± 2, saline = 82.7 ± 5, n = 3, $P < 0.0001$). Likewise in POMC EGFP transgenic mice[2], IP administration of PYY 3-36 resulted in an increased number of arcuate cells positive for c-fos (Figure 2b), compared with saline control animals, Figure 2a and fos co-localisation with POMC EGFP expression was observed, see Figure 2c.

These observations suggested that PYY 3-36 may act via the arcuate nucleus.

EXAMPLE 3

Action of PYY 3-36 and its effects on POMC and NPY circuits in the hypothalamus

In view of the sustained inhibition of food intake and the effects on weight gain following peripheral administration of PYY 3-36, both POMC and NPY hypothalamic messenger RNA (mRNA) were measured using RNase protection assays. A significant decrease in NPY mRNA in response to PYY 3-36 was observed 6 hours post IP injection, compared with saline treated animals (saline = 17.3 ± 2.0, PYY 3-36 = 8.8 ± 1.0, relative optical density units, $P < 0.02$). A non-significant increase occurred in POMC mRNA levels.

EXAMPLE 4

Effect on feeding of PYY 3-36 and of Y2A injected into the arcuate nucleus of rats and the feeding effects of intraperitoneal PYYY 3-36 on wild type and Y2R null mice

To investigate whether peripheral PYY 3-36 inhibits food intake via the Y2R in the arcuate nucleus, an area known to be directly accessible to circulating hormones[4], this hypothesis, PYY 3-36 was injected directly into the arcuate nucleus[12]. Figure 3a shows the results obtained when fasted rats were injected with saline or PYY 3-36 into the arcuate nucleus at the doses indicated in Figure 3a. Post-injection 2 hour food intake was measured.

In rats fasted for 24 hours, food intake was significantly decreased by doses as low as 100 fmol, see Figure 3a, resulting in a similar inhibition to that seen following IP administration.

To establish whether these effects were via the Y2R (the neuropeptide Y Y2 receptor) a Y2R selective agonist[13], N-acetyl (Leu ^{28}, Leu ^{31}) NPY (24-36) [Y2A] was used. Its affinity was confirmed using receptor-binding studies[14] on cell lines expressing the NPY Y1, Y2 and Y5 receptors (Y2 IC_{50} = 1.3 ± 0.2 nM, Y1 IC_{50} > 5000 nM, Y5 IC_{50} > 5000 nM). Intra-arcuate injection of Y2A in rats previously fasted for 24 hours dose-dependently (100 fmol - 1 nmol) inhibited food intake (chow ingested 2 hours post-injection, 0.1 nmol Y2A = 6.2 ± 0.5 g, saline = 8.2 ± 0.6 g, n = 8 per group, $P < 0.05$).

To confirm the anatomical specificity of this effect we injected Y2A (100 fmol - 1 nmol) into the paraventricular nucleus (PVN)[15] of rats fasted for 24 hours and found no alteration of food intake (2 hour post-injection saline = 8.3 ± 0.4 g, 0.1nmol Y2A = 8.0 ± 0.6 g, n = 8 per group).

To further establish the role of the Y2R in the feeding inhibition caused by peripheral PYY 3-36, the effect of PYY 3-36 on Y2R null mice and littermate controls was determined. Figures 3b and 3c show the feeding response to PYY 3-36 in Y2R null mice (Figure 3c)and their wild type littermates mice (Figure 3b). The mice, fasted for 24 hours, were injected with PYY 3-36 at the doses indicated (μg/100g), or saline, and 4-hour cumulative food intake was measured. The results given in Figures 3b and 3c show that the PYY 3-36 dose responsively inhibited daytime feeding in fasted male wild-type mice but no inhibition of food intake was seen in fasted male Y2R null mice.

PYY 3-36 shows a 70% amino acid sequence identity to NPY and acts through NPY receptors[10]. PYY 3-36 is a high affinity agonist at the NPY Y2 receptor[8]. The Y2R is an

inhibitory pre-synaptic receptor and is highly expressed on the arcuate NPY neurons[3], though not on the neighbouring POMC neurons. The Y2R null mouse has been reported to have a mildly obese phenotype and displays increased food intake[11]. Hitherto, no role for Y2R in the acute regulation of feeding had been identified. Our results suggest that peripheral PYY 3-36 inhibits food intake via the Y2R in the arcuate nucleus, an area known to be directly accessible to circulating hormones[4].

EXAMPLE 5

Electrophysiological and neuropeptide responses to PYY 3-36 and Y2A

The electrophysiological response of hypothalamic POMC neurons to administration of both PYY 3-36 and Y2A were investigated. These neurons were identified using mice with targeted expression of green fluorescent protein in POMC neurons[2].

PYY 3-36 disinhibited the POMC neurons, resulting in a significant depolarisation of 19 of the 22 POMC neurons tested, see Figure 4a, which shows the effect of PYY 3-36 (10 nM) on POMC neuron membrane potential (figure representative of recordings from 22 neurons). The results obtained show 10.3 ± 2.1 mV depolarisation, $n = 22$, $P < 0.0003$). At higher PYY 3-36 concentrations an inhibition of POMC neurons was observed (50 nM: data not shown), probably due to activation of NPY Y1 receptors that have previously been shown to hyperpolarize and inhibit POMC neurons[2]. A similar depolarisation was seen with Y2A (8.7 ± 1.8 mV depolarisation, $n = 9$, $P < 0.002$), although no inhibition of POMC neurons was observed at high concentrations of Y2A, due to the greater selectivity of

Y2A for the NPY Y2R. In some neurons the effect of PYY 3-36 was reversed upon washout, but only after a long latency (30 min). We have observed a similar washout of leptin effects upon these neurons.

Figure 4b shows the effect of PYY 3-36 (50 nM) on spontaneous IPSCs onto POMC neurons (figure representative of recordings from 13 neurons). The results obtained show that PYY 3-36 reduced the frequency of inhibitory post-synaptic currents (IPSC) onto all 13 POMC neurons tested (51.9 ± 9.2 % reduction, $n = 13$, $P < 0.0001$), indicating a reduced frequency of GABA release onto POMC neurons. A similar effect was seen with Y2A (44.4 ± 9.3% reduction, $n = 8$, $P < 0.004$) suggesting this effect to be via Y2R. These effects on membrane potential and GABAergic frequency occurred with similar latencies. PYY 3-36 (25 nM) caused a hyperpolarization (5.2 ± 1.16 mV, $P < 0.004$, $n = 5$) of unidentified, but presumably NPY-containing, non-POMC, neurons in the arcuate (data not shown). We have previously shown that there is a tonic GABAergic inhibition of POMC neurons by NPY neurons[2] and these results suggest that PYY 3-36 acts by inhibiting NPY neurons, thus decreasing this GABAergic tone and consequentially disinhibiting POMC neurons.

The effect of Y2A on peptide secretion was also examined using hypothalamic explants[15]. Figures 4c and 4d show αMSH (Figure 4c) and NPY (Figure 4d) released from hypothalamic explants in response to Y2A [N-acetyl-(Leu^{28},Leu^{31})NPY(24-36)]. Hypothalamic slices were incubated with artificial CSF, with or without 50 nM Y2A, for 45 min. The results obtained show that Y2A significantly decreased NPY release, with a concomitant increase in αMSH release from

hypothalamic explants (Fig. 4c & 4d). Taken together these observations suggest that PYY 3-36 modulates both the NPY and melanocortin systems in the arcuate nucleus.

EXAMPLE 6

Effects of PYY 3-36 on appetite and food intake in man

Because of the profound effects of PYY 3-36 on both feeding and weight change seen in rodents shown above, the effects of PYY 3-36 on appetite and food intake in man were investigated.

Twelve healthy fasted, non-obese volunteers (six men and six women, mean age 26.7 ± 0.7 years, BMI = 24.6 ± 0.94 $kg.m^{-2}$ were infused with PYY 3-36 (0.8 $pmol.kg^{-1}.min^{-1}$) or saline for 90 minutes in a double-blind placebo controlled crossover study. PYY 3-36 plasma concentrations increased from mean basal concentration of 8.3 ± 1.0 $pmol.l^{-1}$ to 43.5 ± 3 $pmol.l^{-1}$ during the PYY 3-36 infusion and mimicked post-prandial levels[5, 6]. Post-infusion, PYY 3-36 concentrations returned to basal within 30 minutes.

Figure 5a shows calorie intake from "free-choice" buffet meal 2 hours after infusion with saline or PYY 3-36. The thin lines indicate individual changes in calorie intake for each subject between saline and PYY 3-36 administration. The thick line represents mean change between the two infusions (n = 12).

Figure 5b shows 24 hour calorie intake following infusion with saline or PYY 3-36. Total calorie intake, as assessed by food diaries, is shown for the 24-hour period following either saline or PYY 3-36 infusion.

Figure 5c shows the appetite score (relative scale). Visual analogue scores[17] show perceived hunger during and after infusions.

The PYY 3-36 infusion resulted in a significant decrease in hunger scores[17], see Figure 5c. Calorie intake, during a free-choice buffet meal[18] two hours after the termination of the infusion, was reduced by over a third compared to saline (36 ± 7.4%, $p < 0.0001$), see Figure 5a. There was no effect upon fluid intake and no difference in sensations of fullness or nausea reported by the volunteers (data not shown). PYY 3-36 administration had no effect on gastric emptying, as estimated by the paracetamol absorption method[18, 19], or on plasma glucose (data not shown). There were no effects on plasma leptin or insulin. Analysis of the food diaries revealed a significant inhibition of food intake in the 12 hour period following the PYY 3-36 infusion (saline = 2205 ± 243 Kcal, PYY 3-36 = 1474 ± 207 Kcal). However, food intake during 12 to 24 hour period between the two groups was virtually identical. Overall there was a 33% decrease in cumulative total calorie consumption in the 24-hour period following the PYY 3-36 infusion, see Figure 5b.

These findings demonstrate that infusion of PYY 3-36, matching postprandial levels, caused a marked inhibition of both appetite and food intake in man.

SUMMARY AND CONCLUSION

It has been hypothesized that the cells within the arcuate detect circulating peripheral satiety signals and relay these signals to other brains regions[20]. This is supported by the observation that leptin modifies the activity of

both the POMC and NPY arcuate neurones[2]. We have now shown, through a combination of electrophysiological and hypothalamic explant studies, that the gut hormone, PYY 3-36, can directly influence hypothalamic circuits, resulting in co-ordinate changes in POMC and NPY action. Furthermore, PYY 3-36 administered directly into this brain region reduces food intake. Our data demonstrates that post-prandial levels of PYY 3-36 inhibits food intake in both rodents and man for up to 12 hours. This is in contrast to previously characterised gut-derived 'short-term' satiety signals e.g. cholecystokinin[1, 21], the effects of which are relatively short-lived. The failure of PYY 3-36 to inhibit food intake in the Y2R null mice provides further evidence that PYY 3-36 reduces food intake via a Y2R dependent mechanism. Our results suggest the existence of a novel gut-hypothalamic pathway in the regulation of feeding, involving post-prandial PYY 3-36 acting at the arcuate Y2R. PYY 3-36 appears to be a physiologically important everyday regulator of appetite in man.

The results described above also demonstrate that, by administering PYY, PYY 3-36 or another NPY Y2 receptor agonist, both appetite and food intake can be controlled. Accordingly, PYY, PYY 3-36 and other NPY Y2 receptor agonists may be used inter alia in weight control, in particular to bring about or assist in weight loss or to prevent weight gain, whether for therapeutic reasons as in the treatment of obesity or for "cosmetic" or lifestyle purposes. Conversely, neuropeptide Y Y2 antagonists maybe used in cases where it is desired to stimulate appetite or increase weight, for example, in illness or convalescence, after accidental or surgical trauma, for example, burns, during wasting and anorexia, and in the elderly.

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CLAIMS:

1. A method of reducing or inhibiting food intake, preventing or reducing weight gain, or inducing or promoting weight loss in a subject, which comprises administering PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist to the subject.

2. A method of reducing, suppressing or inhibiting appetite; inducing, increasing, enhancing or promoting satiety and sensations of satiety; or reducing, inhibiting or suppressing hunger and sensations of hunger in a subject, which comprises administering PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist to the subject.

3. A method of treating, reducing or preventing obesity in a subject, which comprises administering PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist to the subject.

4. Use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist for the
manufacture of a medicament for reducing or inhibiting food intake, preventing or reducing weight gain, or inducing or promoting weight loss in a subject.

5. Use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist for the manufacture of a medicament for reducing, suppressing or inhibiting appetite; inducing, increasing, enhancing or promoting satiety and sensations of satiety; or reducing, inhibiting or suppressing hunger and sensations of hunger in a subject, which comprises

administering PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist to the subject.

6. Use of PYY, PYY 3-36 or another neuropeptide Y Y2 receptor agonist for the manufacture of a medicament for treating, reducing or preventing obesity in a subject.

7. A method of increasing, promoting or inducing food intake, promoting or increasing weight gain, or preventing or reducing promoting weight loss in a subject, which comprises administering a neuropeptide Y Y2 receptor antagonist to the subject.

8. A method of increasing, inducing or promoting appetite; reducing, inhibiting or preventing satiety or sensations of satiety; or increasing, inducing or promoting hunger and sensations of hunger in a subject, which comprises administering a neuropeptide Y Y2 receptor antagonist to the subject.

9. A method of treating, reducing or preventing wasting, cachexia or anorexia in a subject, which comprises administering a neuropeptide Y Y2 receptor antagonist to the subject.

10. Use of a neuropeptide Y Y2 receptor antagonist for the manufacture of a medicament for increasing, promoting or inducing food intake, promoting or increasing weight gain, or preventing or reducing promoting weight loss in a subject.

11. Use of a neuropeptide Y Y2 receptor antagonist for the

manufacture of a medicament for increasing, inducing or promoting appetite; reducing, inhibiting or preventing satiety or sensations of satiety; or increasing, inducing or promoting hunger and sensations of hunger in a subject.

12. Use of a neuropeptide Y Y2 receptor antagonist for the manufacture of a medicament for treating, reducing or preventing wasting, cachexia or anorexia in a subject.

13. A method as claimed in any one of claims 1 to 3 and 7 to 9, or use as claimed in any one of claims 4 to 6 and 10 to 12, wherein the subject is a mammal.

14. A method or use as claimed in claim 13, wherein the mammal is a human or a companion animal.

15. A method as claimed in any one of claims 7 to 9, or use as claimed in any one of claims 10 to 12, wherein the subject is a commercially reared animal.

16. A method as claimed in any one of claims 1 to 3, 7 to 9, and 13 to 15 or a use as claimed in any one of claims 4 to 6, 10 to 12 and 13 to 15, wherein the neuropeptide Y Y2 receptor agonist is polypeptide Y (PYY), PYY 3-36, or N-acetyl-(Leu^{28},Leu^{31})NPY(24-36).

17. A method or a use as claimed in claim 16, wherein the PYY or PYY 3-36 is human PYY or PYY 3-36.

18. A method as claimed in any one of claims 1 to 3, 7 to 9, and 13 to 15 or a use as claimed in any one of claims 4 to 6, 10 to 12 and 13 to 15, wherein the neuropeptide Y Y2 receptor agonist is a fragment of PYY or of PYY 3-36, or a

peptide or non-peptide derivative or analogue PYY or of PYY 3-36, which fragment, derivative or analogue has NPY Y2 receptor agonist activity.

19. A method or a use as claimed in any one of the preceding claims, for maintaining any one of more of a desired body weight, a desired Body Mass Index, a desired appearance or good health.

ABSTRACT

APPETITE SUPPRESSION

The use of PYY, PYY 3-36 and other NPY Y2 receptor agonists in the control of weight and appetite, in particular in promoting weight loss, reducing weight gain, decreasing appetite and hunger, and increasing satiety is disclosed. Such agents can be used in the treatment of obesity.

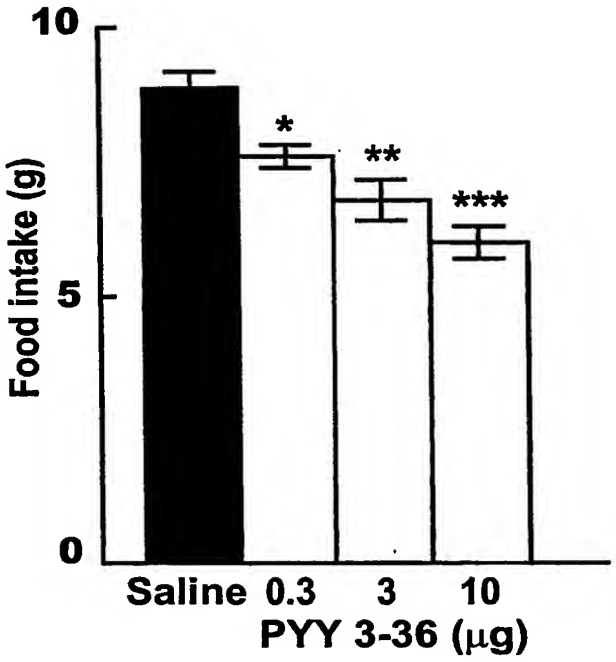


Figure 1a

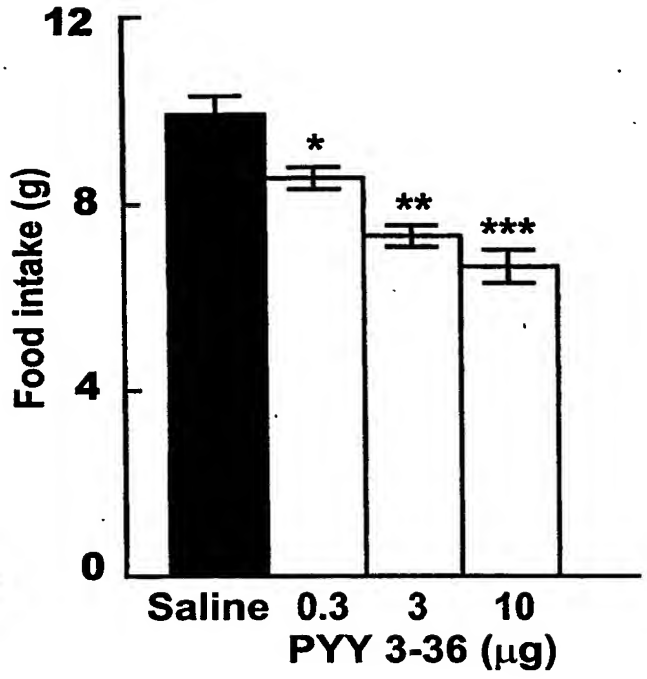


Figure 1b

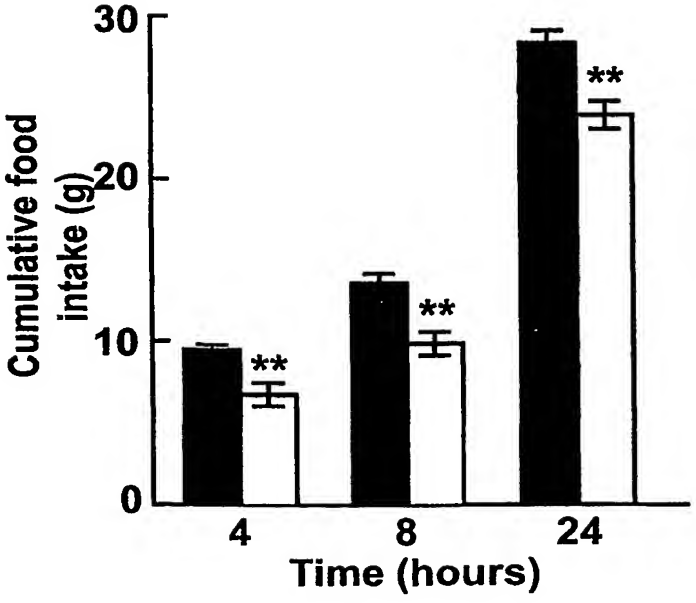


Figure 1c

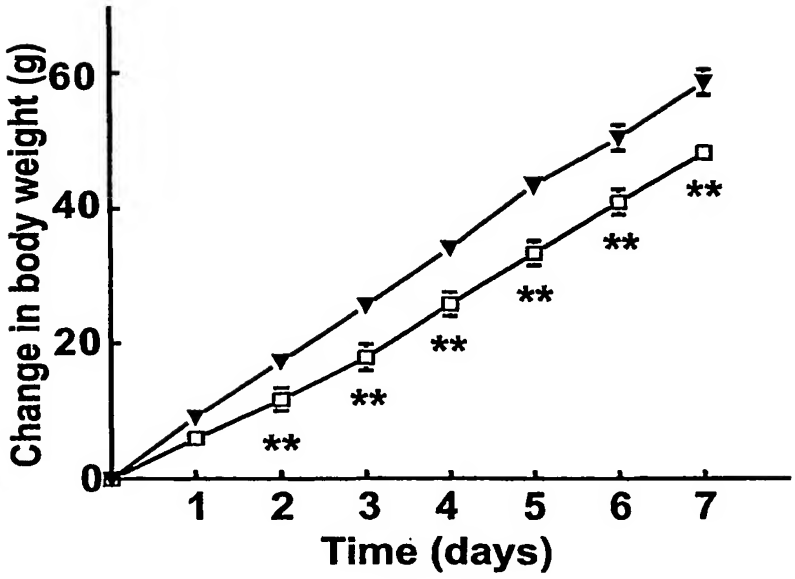


Figure 1d

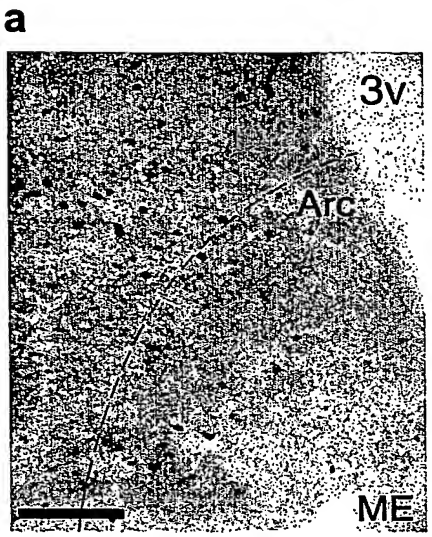


Figure 2a

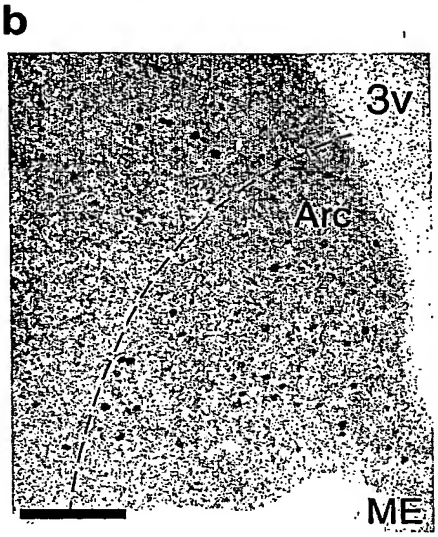


Figure 2b

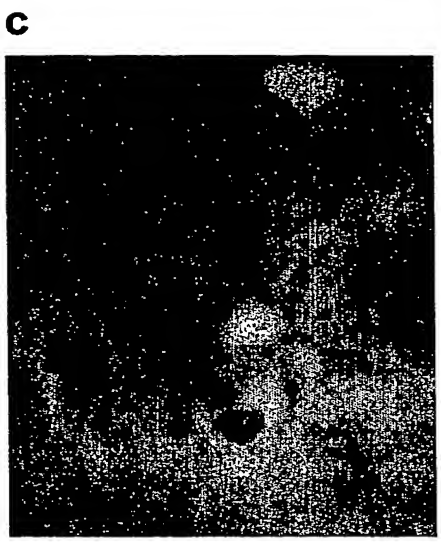


Figure 2c

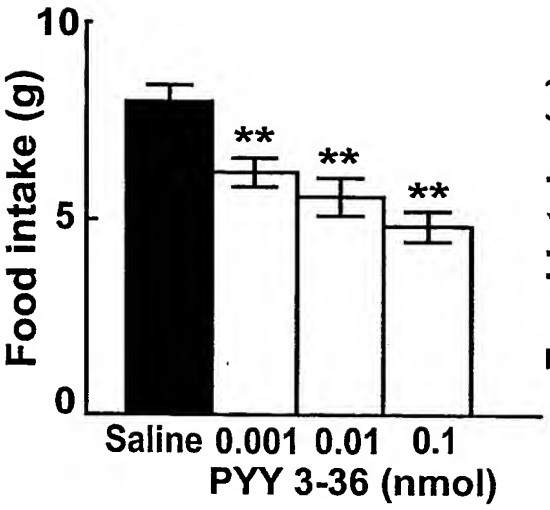


Figure 3a

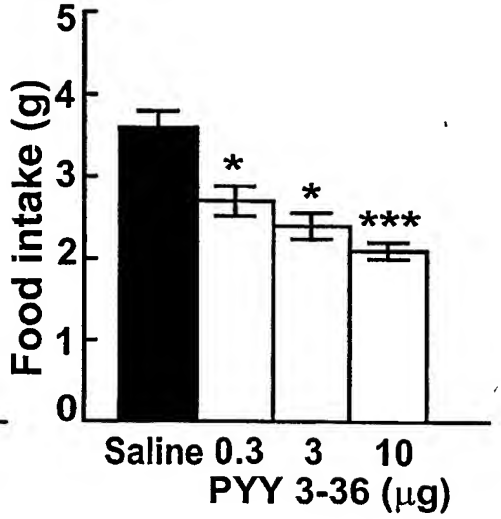
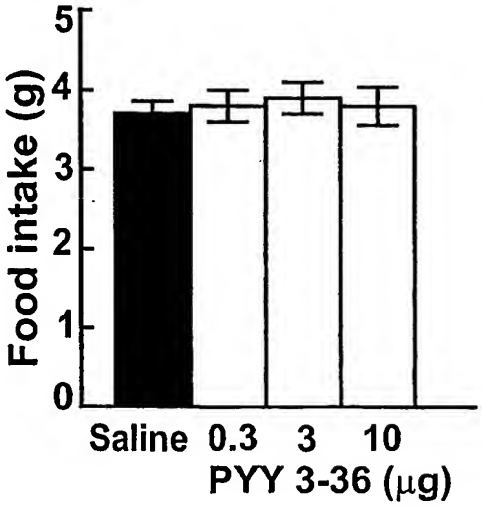


Figure 3b



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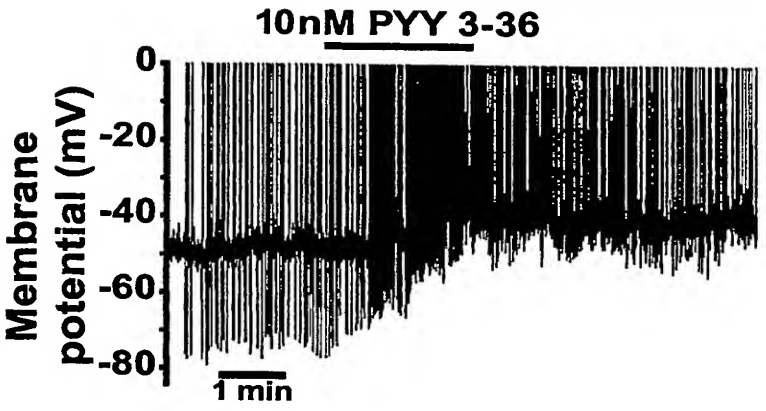
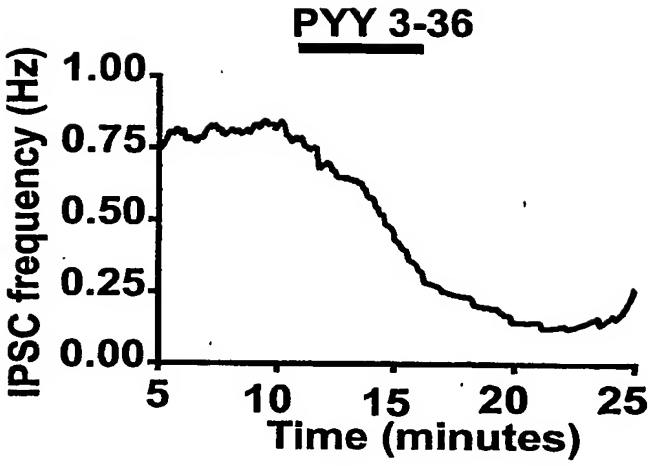


Figure 4a



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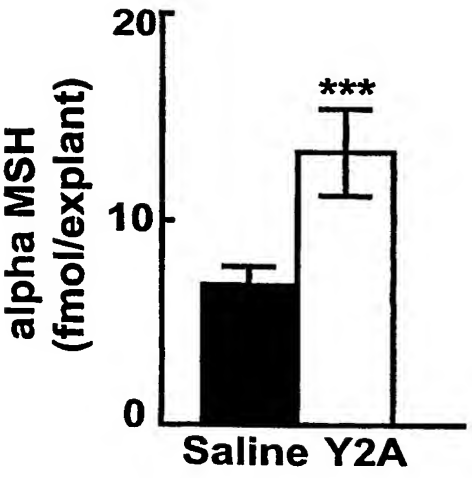


Figure 4c

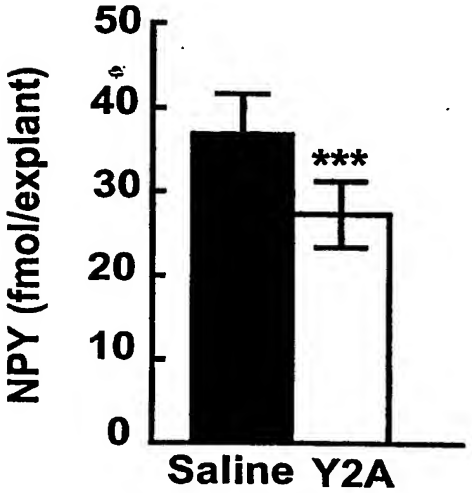


Figure 4d

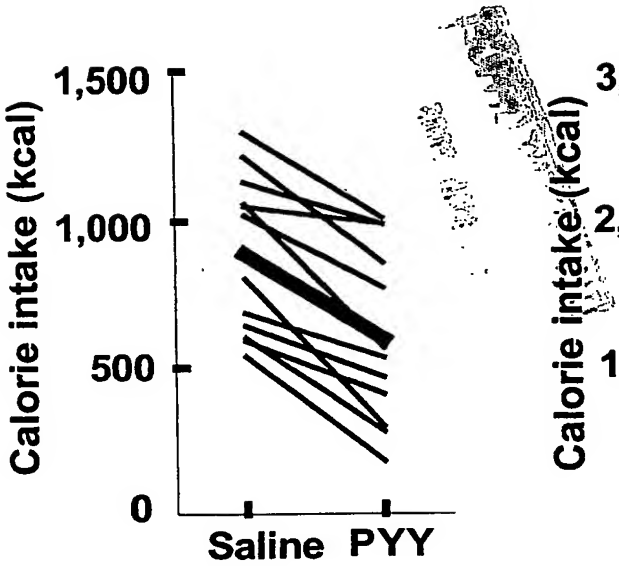


Figure 5a

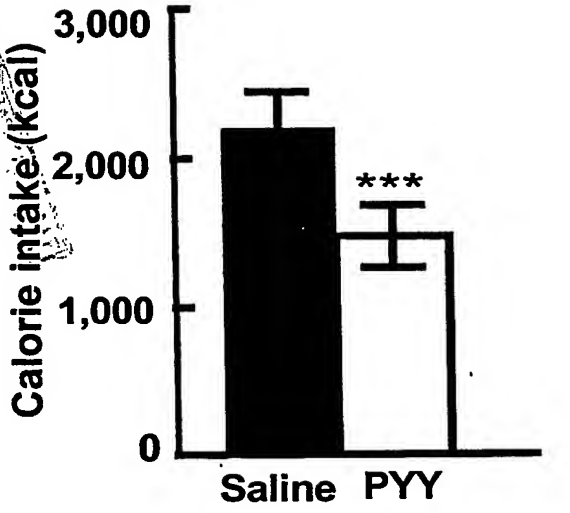
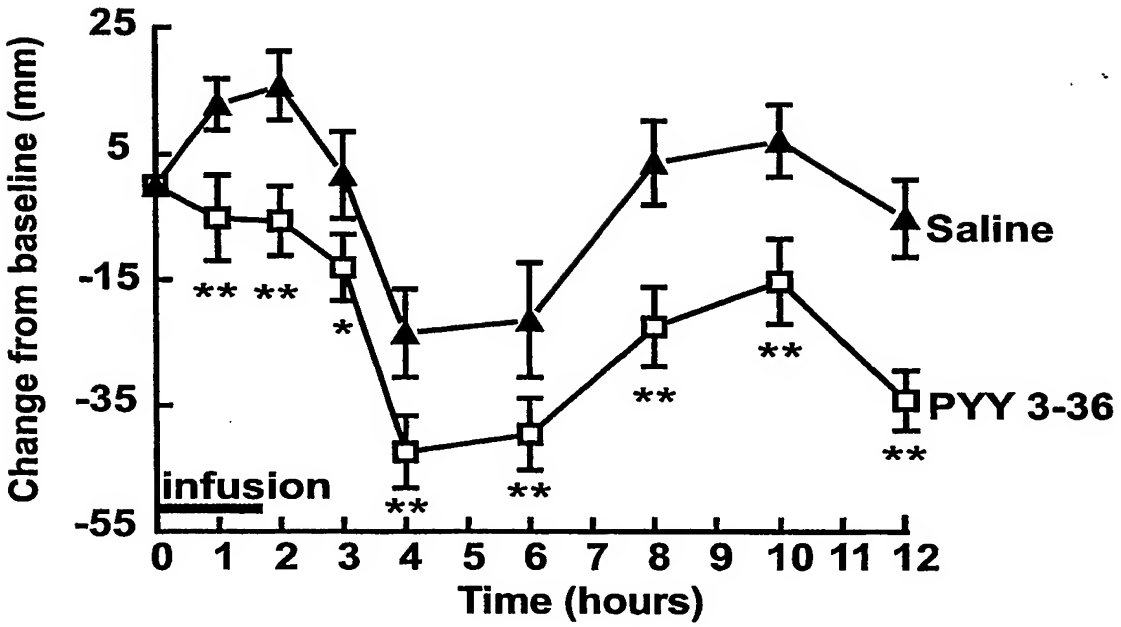


Figure 5b



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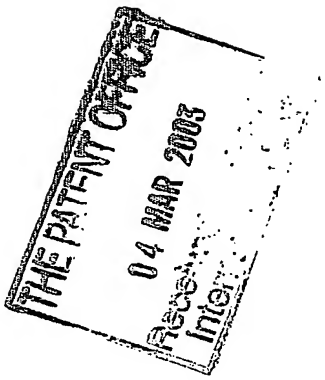
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